STUDIES ON BRITISH PYRENOMYCETES

I. THE LIFE HISTORIES OF THREE SPECIES OF *CEPHALOTHECA* FUCK.

BY

CHAS. G. C. CHESTERS

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STUDIES ON BRITISH PYRENOMYCETES

I. THE LIFE HISTORIES OF THREE SPECIES OF *CEPHALOTHECA* FUCK.

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(With Plates XI and XII and 5 Text-figures)

I. Introduction

It is proposed to publish the results of a series of investigations by pure culture methods of the life histories of selected species of Pyrenomycetes, paying particular attention to the development of the imperfect stages and to the development and cultural requirements of the ascophorous stages where these are obtained. Pyrenomycetes are known mainly in their perfect stage, and precise information of the imperfect condition is often lacking. Generic and specific diagnoses which may later be used in an attempt to indicate the evolution of the group as a whole should be on a wide comparative basis. The immediate necessity appears to be to provide detailed accounts of the development of the spore-bearing structures of definite species.

Winter (10), following Fuckel, retained the genus *Cephalotheca* in his suborder Perisporieae of the Pyrenomycetes, but Fischer (3) placed it in the Aspergillaceae which he treated as a family of his new order, Plectascineae, definitely removed from the Pyrenomycetes. Von Höhnel (5) suggested that the genus should be transferred to a separate family, Cephalothecaceae, which, though related to Aspergillaceae, contained types with a specialised perithecial structure for the liberation of the ascospores. In this he was followed by Nannfeldt (7). It is not intended to consider here the validity of Pyrenomycetes as a specialised class within the Ascomycetes; in this series of papers the word is used in the sense employed by Winter.

In order that the systematic position adopted may be clear, a summary is given below, and the reasons which led to this will be discussed in later sections.

Cephalotheca Fuck. *Symb. Myc. Nachtr.* I (1871), 9 (297).

Syn. *Crepinula* O. Kuntze, *Rev. Gén.* Pl. II (1891), 850.
Fairmania Sacc. in *Ann. Mycol.*, IV (1906), 276.
Aposphaeriopsis Died. *pro parte* in *Ann. Mycol.* XI (1913), 44.
Fragosphaeria Shear in *Mycologia*, XV (1923), 124.

(1) *C. sulfurea* Fuck. Type species, *loc. cit.*
(2) *C. reniformis* Sacc. & Therry apud Sacc. in *Mich.* II (1881), 312.
Syn. *Fairmania singularis* Sacc. *loc. cit.*
Aposphaeriopsis fusco-atra Died. *loc. cit.*
(3) *C. purpurea* (Shear) comb.nov.
Syn. *Fragosphaeria purpurea* Shear, *loc. cit.*

II. Comparison of material collected with authentic material and original descriptions

Each field collection of the species investigated from which monospore cultures were obtained was compared either with authentic material or, failing this, with the original diagnosis. All the material which I have examined or which has been examined on my behalf is given below.

(1) *C. sulfurea* Fuck.

Authentic material. Fuckel, Fungi Rhenani, 2313, preserved in the Herbaria of: (1) The British Museum, general fungus collection. (2) C. E. Broome; incorporated in the British Collection in the British Museum. (3) The Pathological Collection, Bureau of Plant Industry, Washington, U.S.A.* (4) The Royal Botanic Garden, Kew.† (5) The Botanischer Garten und Museum, Berlin Dahlem (specimen kindly loaned by Prof. Dr E. Ulbrich).†

British Material. C. E. Broome's material was collected at: (*a*) Bristol, April 1876, and (*b*) Henbury, October 1879.

Material used for isolation. Collected by W. B. Grove, on garden cane, Birmingham, 1931. Preserved in my herbarium, No. 4.

(2) *C. reniformis* Sacc. & Therry.

No authentic or other material available for comparison. (The type specimen is cited by G. Gola (1930) in *L'erbario Micologico di P. A. Saccardo*, p. 165, as being preserved in Saccardo's herbarium.)

Material used for isolation was collected on dead beech, Richmond Park, Surrey, September 1931 (No. 1167) and June 1932 (No. 1223) by Mr E. W. Mason, and on dead oak, Birmingham, June 1933 (No. 5) and February 1934 (No. 67) by myself.

(3) *C. purpurea* (Shear) comb.nov.

Authentic material. In Herbarium C. L. Shear: pure culture tube No. 4821, on corn-meal agar (cf. Shear *loc. cit.* p. 125).‡

Material used for isolation was collected by Mr E. W. Mason on

* C. L. Shear, *in litt.*
† Without any discernible fungus, E. W. Mason, *in litt.*
‡ I am indebted to Dr Shear not only for comparing with his type specimen isolations from collected material sent to him, but also for providing me with a slide from his specimen.

dead beech, Richmond Park, Surrey, July 1932 (No. 1228), and on dead oak, April 1933 (No. 12 of my herbarium).

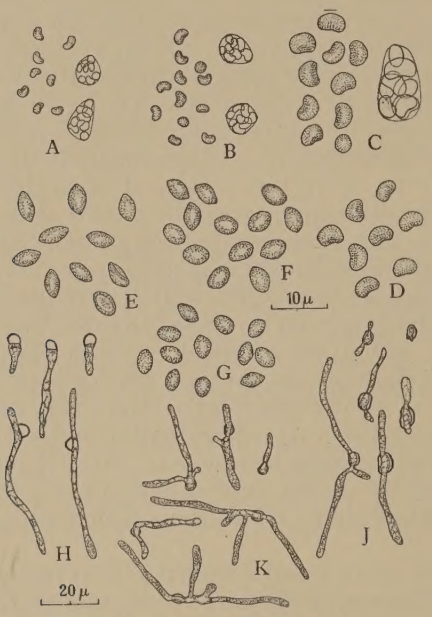
(1) *C. sulfurea* Fuck.

The genus was founded on *C. sulfurea* and *C. trabea*. The former was placed first, has been recognised since on more than one occasion, and is universally recognised as the type species.

The collection made by W. B. Grove consists of discrete groups of astomous perithecia of various ages surrounded by a sulphur-coloured villus of rather flexuose hyphae (Pl. XI, fig. 1). The young perithecia are totally embedded in the general mycelium and are individually covered by sulphureous hyphae (Pl. XI, fig. 1 A). The mature perithecia (250–500μ in diameter) are glabrous at the apex, black, and have a series of definite suture lines regularly disposed over their surface (Pl. XI, fig. 1 B). The polygonal areas delimited by these sutures are often torn apart, revealing a mass of brown ascospores (Pl. XI, fig. 1 C). The oldest perithecia are completely shattered and only a basal cup remains in which the ascospores are loosely held together in small clusters (Pl. XI, fig. 1 D). The perithecia, though embedded in mycelium, are not immersed in the substance of the cane. The ascospores, which are elliptical to ovate (Text-fig. 1 E) and individually of a light brown, measure 4·5–6 × 3–4μ (mostly 6 × 4μ). Asci attached to ascogenous hyphae were not observed in this material, but Fuckel (*loc. cit.*) describes the asci as forming globose clusters at the ends of branched hyphae. Von Höhnel(5) stated that the perithecia of this species, in addition to being fragile, break up along definite suture lines such as those which have been described above.

The two specimens of Fungi Rhenani, No. 2313, in the Herbarium of the British Museum, bear numerous groups of young and adult perithecia enclosed within a definitely sulphureous villus. The mature, but unshattered, perithecia are from 330 to 500μ in diameter, and in both specimens perithecia having sutures can be observed. The ascospores are elliptical or ovoid (Text-fig. 1 F) and measure 4–6 × 3–4μ. The characteristic size appears to be 6 × 4μ, and no ascospores longer than 6μ have been observed. Hence Fuckel's original measurement of 6–7 × 3μ is in need of some slight emendment. The specimen in the Pathological Collection of the Bureau of Plant Industry, Washington, was examined by Dr C. L. Shear who states (*in litt.*) that: "The perithecia are covered with a sulphur-colored layer of hyphae which seems to bear small hyaline globular conidia. Whether this is really genetically related to the perithecia or not is a question.... The perithecia when free break up in much the same manner as in *Fragosphaeria*, and the wall is of the same characteristic structure, and the spores brown. Fuckel's specimen is labelled on Oak."

The British material of *C. sulfurea* in the Broome Herbarium in the British Museum consists of two separate collections, that of October 1879 having young perithecia, some of which show suturing of the wall and all of which are embedded in the characteristic sulphur-coloured mycelium; and that of April 1876 having fully mature and often shattered perithecia with ascospores which measure from 4 to 5·5 × 2·5 to 3μ (Text-fig. 1 G). The latter material is that from which



Text-fig. 1. Ascospores of species of *Cephalotheca*. A, *C. purpurea* (Shear's culture tube No. 4821). B, *C. purpurea* (Mason, No. 1228). C, *C. reniformis* (Mason, No. 1223). D, *C. reniformis* (Mason, No. 1167). E, *C. sulfurea* (Chesters, No. 4). F, *C. sulfurea* (Fung. Rhen. No. 2313). G, *C. sulfurea* (Berkeley & Broome, No. 1729). H–K, germinating ascospores (H, *C. sulfurea*; J, *C. reniformis*; K, *C. purpurea*).

the illustrations published in the *Annals and Magazine of Natural History* (Pl. XII, fig. 1) of 1878 were drawn ((1), p. 30). Berkeley and Broome observed the asci of this species but referred to them (*loc. cit.* p. 30) as "Sporangia"; in this they were followed by Cooke (*Grev.* VI (1878), 128).

The reference which Shear makes to "small hyaline globular conidia" associated with the sulphur-coloured mycelium about the perithecium is of interest. Similar conidia are present in the specimen of Fungi Rhenani, No. 2313, in the British Museum. They measure 3–4 × 3μ and occur in chains of two to several spores, but they have

not been observed attached to mycelium. They are present in considerable numbers in the British collection of Broome of 1879 which contains only immature perithecia. They are not present on the collection of W. B. Grove, but, as will be described later, they are formed on the mycelium produced from the germinated ascospores of this collection.

The collection of *C. sulfurea* from which monospore cultures were obtained agrees precisely as regards the mycelium, perithecia and ascospores with the authentic material of the species and with the previous British collections. Further, conidia present upon authentic material and also on one previous British collection correspond exactly with those produced in culture.

(2) *C. reniformis* Sacc. & Therry

No authentic material of this species was available for examination and comparison, but all the collections of the fungus which have been made agree with the authors' description (*loc. cit.* p. 312), which may be translated thus:

"Perithecia gregarious, nestling among the cortical fibres or at length superficial, globose, mouthless but sometimes with a small apical papilla, later frequently collapsing and becoming cupulate, fuscous black in colour, $\frac{1}{3}$–$\frac{1}{4}$ mm. in diameter; at first clothed all over with filiform fuliginous hairs 2μ in diameter, afterwards becoming glabrous except at the base, with a somewhat thick, fuscous, small celled, parenchymatous texture; asci either globose, 10μ in diameter, or ellipsoid 15×14–12μ, evanescent, eight-spored, ascospores conglobate, reniform, 4–5×3–$3{\cdot}5\mu$, at first olivaceous, later becoming slightly fuliginous.

"Growing in the decorticated branches of decayed Poplar, Lyon, May 1880. Easily distinguished from other species by the small reniform ascospores."

The several collections of this species which I have examined and from which monospore cultures have been obtained all exhibit small perithecia scattered throughout the decayed wood. On beech wood they are mainly clustered round the cavities left by the destruction of medullary ray tissue (Pl. XI, fig. 2), while on oak they are either dispersed throughout the wood or occur in dense masses in, and along, the walls of channels made by a boring beetle. Uncovered by any mycelial investment, the mature perithecia appear as black, shining spherical structures (Pl. XI, fig. 6), 250–350μ in diameter, showing no obvious suturing of the carbonaceous wall. When shattered, the lower part of the wall usually remains intact as a cup within which lies the rather brown powdery mass of spores (Pl. XI, fig. 3). If the perithecia are slowly dried, the splitting of the wall can be observed to take place indiscriminately so that irregularly shaped areas from

the distal portion of the perithecium are set free. The ascospores (Text-fig. 1 C and D), which are held together in clusters, are definitely reniform, light brown and $4{\cdot}5–5{\cdot}0 \times 3{\cdot}0–3{\cdot}8\mu$. The perithecia, unlike those of *C. sulfurea*, are attached to the substratum by a mass of dark brown hyphae radiating from the base of the fruit body and penetrating into the wood.

The finding of this species upon beech at Richmond constitutes the first record of the species in Britain. Since then it has been observed on several occasions at Richmond and Birmingham. In every case the wood has been attacked by a boring beetle, and it is quite possible that the fungus is spread by the beetle.

(3) *C. purpurea* (Shear) comb.nov.

The collection of this species on dead beech at Richmond in 1932 represents its discovery in Britain and also the first field collection on the host plant. The material upon which the original description is based (Shear, *loc. cit.*) was obtained in culture as a contaminant of plates poured for the isolation of *Pilacre Petersii* from beech wood. The Richmond material bears both superficial and immersed perithecia, the latter being situated along the air spaces produced by the decay of the medullary rays. The wood is generally stained brown or brownish purple in the vicinity of the perithecia. These are dark in colour, usually black, and when mature show marked suturing of the wall (Pl. XI, fig. 4). Upon dry wood the majority of the perithecia are shattered (Pl. XI, fig. 7) to such an extent that only a flattened plate of loosely connected polygons of wall tissue remains. This may have a few ascospore clusters scattered over its surface (Pl. XI, fig. 5). Although the base of the perithecium may be slightly embedded in a superficial mycelium or in the rotted wood, it is never definitely attached to the substratum as are the perithecia of *C. reniformis*. In this it resembles *C. sulfurea*. The collection on oak has younger perithecia, and these are separately covered by a weft of purple-coloured hyphae and the walls are also similarly tinted. The vestment of hyphae in this case is soft and not strictly comparable with the sulphureous villus of *C. sulfurea* where the individual hyphae are thickened and flexuose. The asci (*vide* Table I) are subglobose to globose (Text-fig. 1 B) and are arranged in clusters, and although they are evanescent the eight broadly bean-shaped ascospores (Text-fig. 1 B) remain grouped together even after the perithecial wall is shattered. The ascospores measure $2{\cdot}5–3{\cdot}0 \times 1{\cdot}5–2{\cdot}0\mu$ and are light brown in mass but individually more or less hyaline. By itself this species could legitimately be considered as perhaps belonging to the "Hyalosporeae" where in fact it is placed in Clements and Shear, *Genera of Fungi*.

Cultures of mature perithecia were sent to Dr Shear who was so good as to compare them with his type specimen (Pl. XII, fig. 2), and

also to send me a slide of perithecia and ascospores prepared from his type specimen (Pl. XII, fig. 3 and Text-fig. 1 A). He states that: "Your dried culture... I should say is undoubtedly our *F. purpurea*" (*in litt.*). As will be seen from Table I, there is no significant difference between the measurements of the American and British materials of this species. Referring to the generic status of *Cephalotheca sulfurea* and *Fragosphaeria purpurea* Dr Shear states (*in litt.*) that: "At present, I am inclined to think that so far as the genera are concerned they are the same...."

Table I. *Measurements of perithecia and spores of species of* Cephalotheca.

Specimens examined	Perithecia	Asci
C. sulfurea:		
(1) Exsicc. Fung. Rhen. No. 2313	330–500μ	10μ diam.
(2) Exsicc. Berkeley and Broome No. 1729, in Herb. Brit. Mus.	—	—
(3) Exsicc. Chesters No. 4	250–500μ	8–11μ diam.
C. reniformis:		
(1) Measurements from *Syll.* 1, p. 37	250–330μ	15×14–12 or 10μ diam.
(2) Exsicc. Mason No. 1167	250–350μ	11×8–10 or 10–11μ diam.
C. purpurea:		
(1) Measurements from Shear (*loc. cit.* p. 124)	—	4–6μ diam.
(2) Exsicc. Mason No. 1228	300–350μ	5·0–6·5μ diam.

Specimens examined	Ascospores	Conidia
C. sulfurea:		
(1) Exsicc. Fung. Rhen. No. 2313	4–6×3–4μ	3–4×3μ
(2) Exsicc. Berkeley and Broome No. 1729, in Herb. Brit. Mus.	4–4·5×2·5–3μ	3–4×2·0–2·5μ
(3) Exsicc. Chesters No. 4	4·5–6×3–4μ, mostly 6×4μ	4·5–5×2–3μ (cultures)
C. reniformis:		
(1) Measurements from *Syll.* 1, p. 37	4–5×3·0–3·5μ	—
(2) Exsicc. Mason No. 1167	4·5–5×3·0–3·8μ	4–5×1·5–2μ (cultures)
C. purpurea:		
(1) Measurements from Shear (*loc. cit.* p. 124)	2·25–2·75×1·5–2μ	3–4×1·0–1·5μ
(2) Exsicc. Mason No. 1228	2·5–3·0×1·5–2μ	3–4×1·0–1·5μ

The comparisons made in the foregoing pages appear to me to indicate that the species under investigation can be identified with previous descriptions and material of three species, two of which are already recognised as belonging to *Cephalotheca*. The life histories of the three species will indicate similarities which necessitate their common treatment in a single genus.

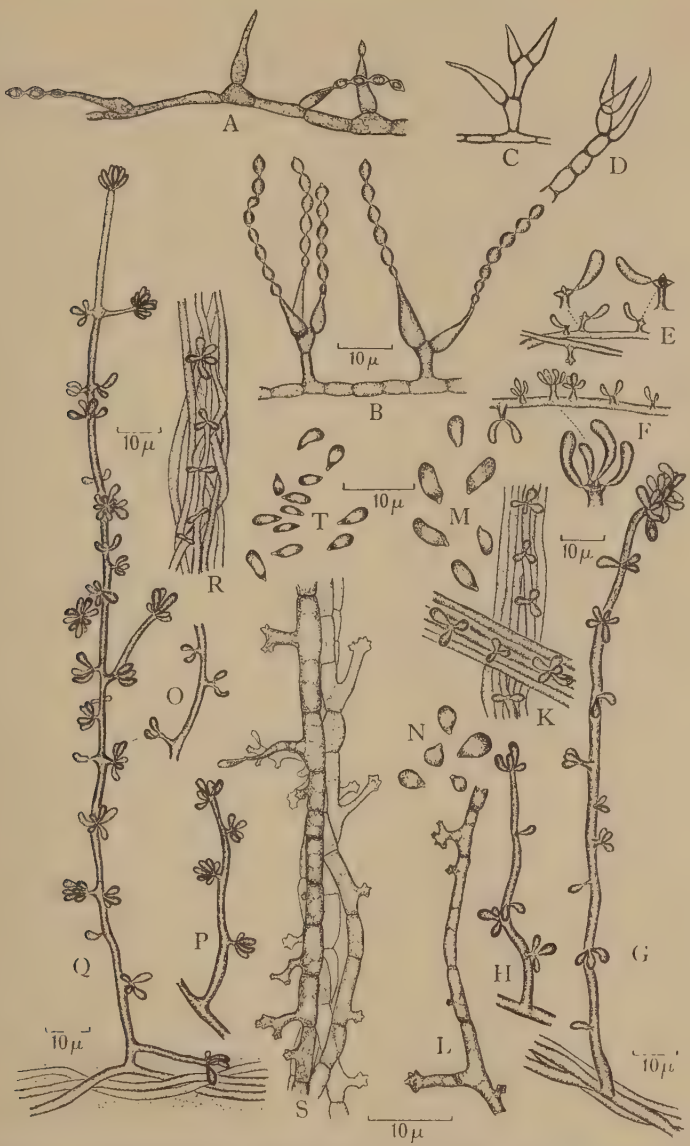
III. The life histories

(*a*) *The conidial stage*

The freshly discharged ascospores germinated easily on a variety of media, but the species show marked differences in the optimum temperature for germination. Those of *C. sulfurea* have an optimum temperature between 16 and 18° C., those of *C. reniformis* between 20 and 26° C., and those of *C. purpurea* between 22 and 26° C. In each case the germ tubes are produced from the poles of the spore (Text-fig. 1 H–K) and the first lateral branch is formed near to the ascospore. Conidia are developed within two to four days after germination. The conidial stage appears earliest in *C. purpurea* and latest in *C. sulfurea*. In each species the first conidia are borne upon conidiophores which are simpler than those present on mature mycelium.

(1) *C. sulfurea.*

The conidia are formed on simple, erect, unicellular branches which are at first of an even thickness throughout their length, but during growth the apical part becomes attenuated and the whole forms a simple flask-shaped cell (Text-fig. 2 A). The first spore is formed as a distal swelling which remains in open communication with the hypha until a second, and lower, spore is differentiated. The wall between the two spores now contracts so that the protoplasmic continuity is severed and a small bridge of wall material separates the two spores. This basipetal formation is repeated and a chain of spores of some considerable length is produced (Text-fig. 2 B). The flask-shaped conidiophore must be regarded as a phialide producing serial phialospores(6) which for a time remain in contact with one another. The conidia are liberated either in units or in small linear series; they never collapse into a sticky mass. The conidia are small, uninucleate, ovate structures (*vide* Table I) which germinate easily upon a variety of media or in tap water. Simple phialides occur on the young mycelium and on aerial parts of the old mycelium, but the surface plexus of hyphae produces more complex structures (Text-fig. 2 B) which may be immersed, and are reminiscent of a rather lax penicillioid growth. On these the phialides arise from branches which may be one or more cells in height (Text-fig. 2 B–D), with an apical group of two or more phialides and lateral phialides, either singly or in groups, throughout their length (Text-fig. 2 C). The very considerable, widespread spore production gives the adult mycelium a white or yellowish powdery appearance. The colour varies on different media and at different temperatures. It is most intense on oat agar containing sucrose at 18° C. No pigmentation of the medium occurs.



Text-fig. 2. A–D, phialides and phialospores of *C. sulfurea*. E–N, fertile hyphae and radulospores of *C. reniformis*. O–T, fertile hyphae and radulospores of *C. purpurea*. For further explanation see text.

(2) *C. reniformis.*

The conidia, which are falcate and 4–5 × 1·5–2μ in size (Text-fig. 2 M), are always borne upon minute papillate processes (Text-fig. 2 E). In their simplest form these arise singly on the cells of the superficial and aerial mycelium (Text-fig. 2 G and H); in their most complex form they are arranged in masses over the ends of swollen lateral projections (Text-fig. 2 F–L). A septum may very occasionally occur at the base of these projections, thus forming a simple unicellular conidiophore. In this latter case a definite spiral arrangement can be traced in the order of production of the papillae (Text-fig. 2 K and L). This type of spore formation was differentiated among others as a "radula* spore form" by Mason (6). In the present state of our knowledge it would be unwise to compare directly this spore form with the phialospores of *C. sulfurea*. A relevant case may be cited from *Botrytis cinerea*, where the "Botrytis" spore is a higher development of the radula spore type shown by *C. reniformis*, and phialospores comparable to the conidia of *C. sulfurea* are represented by the so-called microconidia. On most agar media the mycelium of *C. reniformis* consists of a matted surface growth of interwoven strands of hyphae producing abundant conidia which give the mycelium a somewhat waxy appearance and cream-like colour. As the medium dries out, or upon hard agar or wood, superficial rope-like strands develop which bear upright fertile hyphae of varying length (Text-fig. 2 G and H). This type is floccose in appearance.

(3) *C. purpurea.*

The conidial condition of this species was described by Shear (*loc. cit.* p. 124) as: "Thin, white, effuse, much branched; conidia formed on somewhat enlarged, elongated, and roughened ends of the alternately branched fertile hyphae (Pl. XII, fig. 4), oblong-elliptic, inequilateral or suballantoid, non-septate, hyaline, 3–4 × 1–1·5μ." The fertile hyphae of isolations from British material only occasionally branch in a regularly alternate manner (Text-fig. 2 P). The branching of large fertile hyphae is very irregular (Text-fig 2 Q). The genesis of the individual conidium (Text-fig. 2 O–S) is precisely similar to that of *C. reniformis*, and though somewhat smaller (see Table I), it has the same shape (Text-fig. 2 T). The cultures are more flocculent than those of *C. reniformis*, due to the more extensive development of aerial mycelium. On microscopic characters alone it would be impossible to differentiate the conidial stage of *C. purpurea* from that of *C. reniformis*. The macroscopic appearance of the cultures is certainly different in a degree, but not to such an extent as would allow one without previous knowledge of the perithecia connected with each species to consider them specifically distinct.

* *radula* (Latin)=nutmeg grater.

(*b*) *The ascophorous stage*

Perithecia are only abundant when the factors which influence the growth of each species are at their optimum value. Of these factors the composition of the medium, the humidity of the atmosphere and the temperature are the most important, provided that the reaction of the medium falls within *p*H 6·0–7·0. The most favourable media are maize or oat paste agar and 2 per cent. malt agar. Synthetic media containing not less than M/250 maltose and $NaNO_3$ or KNO_3 as the source of nitrogen support the formation of perithecia by *C. reniformis* and *C. purpurea* but not by *C. sulfurea*. Maltose is a better source of carbohydrate than sucrose.

Perithecia of *C. reniformis* and *C. purpurea* develop abundantly upon fresh, autoclaved wood of beech, oak and ash, but only sparsely upon rotted specimens of these woods or specimens which have been subject to water extraction and partial chlorination. It is difficult to develop the perithecia of *C. sulfurea* either on fresh or rotted wood. The optimum range of atmospheric humidity lies between one-eighth and five-eights of full saturation, and perithecia are usually more plentiful in drier conditions. A very clear optimum range of temperature exists for each species. Linear growth of *C. sulfurea*, though never extensive, is greater between 16 and 18° C., and is negligible at 24° C. Perithecia have so far only been developed upon hard malt agar and oat paste agar between 16 and 18° C. with an air humidity of one-eighth to one-quarter saturation. The relative linear growth and perithecial formation of *C. reniformis* and *C. purpurea* is given in Table II, which shows that the optimum temperature for the former is slightly lower than for the latter. This is quite independent of the medium used.

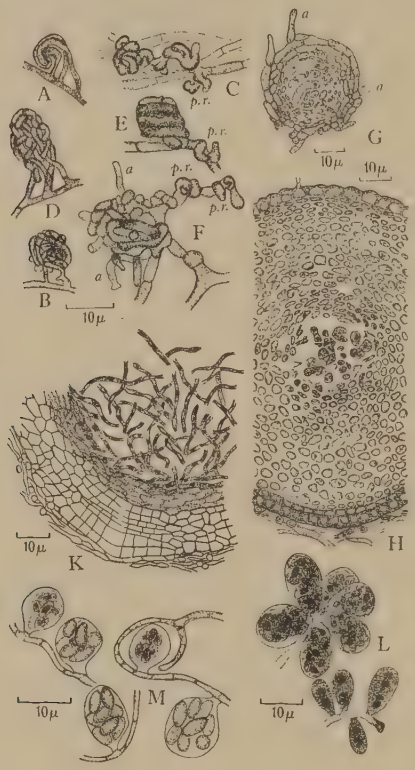
Table II

Temperature (malt agar)	Linear growth as a % of the optimum value		Relative abundance of perithecia	
	reniformis	*purpurea*	*reniformis*	*purpurea*
13	68	50	+	0
19	100	87	+++	+++
24	99	100	++	++++
29	85	99	0	++

+ = few perithecia; ++++ = abundant perithecia.

The perithecial rudiments of the three species are similar and consist of two (Text-fig. 3 C) or, sometimes, three (Text-fig. 3 A) small upright branches which coil loosely round one another to produce an irregular or spherical structure (Text-fig. 3 B–F). They are scattered over the surface mycelium of *C. reniformis* and among the aerial hyphae as well in *C. sulfurea* and *C. purpurea*. As yet it is impossible to say whether the fundaments should be regarded as func-

tional sexual organs. No fusion has been observed between the coiled hyphae. These of *C. reniformis* and *C. purpurea* (Text-fig. 3 A, B, D) are similar and differ in their more delicate construction from those of *C. sulfurea* (Text-fig. 3 C, E, F). The coiled hyphae soon become surrounded by branching filaments which arise from their base (Text-



Text-fig. 3. Stages in the development of the perithecia of *C. purpurea* (A and D), *C. reniformis* (B) and *C. sulfurea* (C, E and F). G, longitudinal section through a young perithecium of *C. reniformis* showing the central spiral hypha and young attachment hyphae (*a*). H, an older perithecium of *C. reniformis* showing the central ascogenous hyphae. K, a sector of the perithecium of *C. sulfurea* showing the loosely woven ascogenous hyphae. L, asci of *C. reniformis*. M, asci of *C. sulfurea*.

fig. 3 B, D). These sterile filaments form the wall of the perithecium and soon become brown. Thus the perithecium is entirely derived from the further growth of two or three fundamental hyphae. At this time, when the outer wall layers are first clearly defined, the perithecium of each species is similar in appearance (Text-fig. 3 G). A double layer of brown, thick-walled cells surrounds a mass of inter-

woven hyphae, in the centre of which lies a single spiral hypha in whose dense finely granular protoplasm prominent nuclei are embedded. The examination of numerous perithecia which were cleared and then stained in erythrosin indicates definitely that only a single hypha is concerned. Subsequent development varies according to the species.

(1) *C. reniformis.*

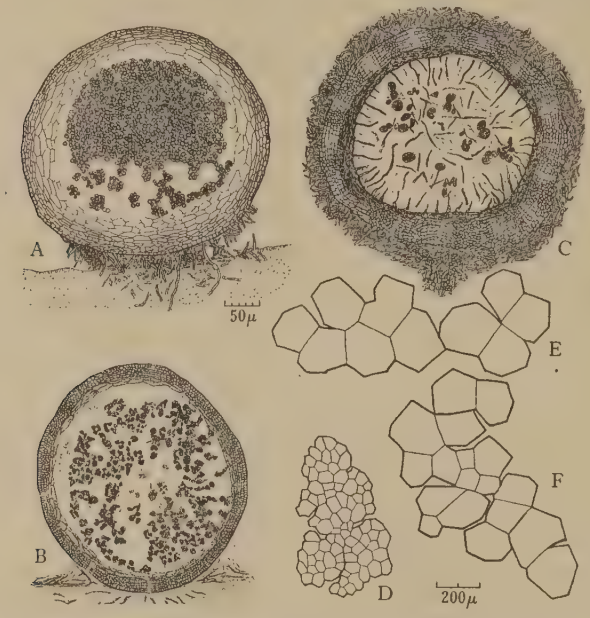
As the perithecium grows, a spherical mass of hyaline pseudoparenchymatous tissue is formed within the dark brown or black wall layers. In the centre of this lie a number of hyphae which stain deeply and often contain paired nuclei (Text-fig. 3 H). They are ascogenous hyphae and are derived from the original spiral hyphae. Increasing in number they become interpolated between the cells of the ground tissue which is ultimately destroyed except for a thin zone adjacent to the outer indurated wall. During this the development of clusters of asci at the apices of the ascogenous hyphae (Text-fig. 3 L) begins and continues until the centre of the perithecium is almost completely filled. The asci are spherical or elliptical, and, being entirely occupied by ascospores, appear sessile. They are evanescent and cannot usually be found in shattered perithecia. They may even have disappeared before normal rupture of the perithecial wall occurs. The eight ascospores are definitely reniform from the time the spore wall is formed, and though at first hyaline they gradually become olive brown and finally fuliginous.

The mature perithecium (Text-fig. 4 A) is a spherical, black, glabrous structure firmly attached to the substratum by brown thick-walled hyphae which are produced at an early stage in development as outgrowths from the hyphae forming the wall (Text-fig. 3 G, *a*). This is about three to five cells in thickness, and in surface view appears to consist of small regular plates (Text-fig. 4 D) of indurated cells separated by a network of thinner walled elements which tear when the perithecium ruptures. As the perithecium dries, and prior to the actual fragmentation, the apical portion generally collapses to form a cup-shaped depression. This was noted by Saccardo in his diagnosis of the species. An apical cap may then be forced off or the whole perithecium may gape in an irregularly two-lipped manner. The basal attached portion usually remains as a cup and is filled with a brown powdery mass of ascospores.

(2) *C. purpurea.*

In this species the development of the ascogenous hyphae and asci (Text-fig. 3 L) is similar to that in *C. reniformis*, except that the asci are smaller and more numerous. The ascospores are bean-shaped, and though ultimately yellowish they remain hyaline for a consider-

able period. The wall of the mature perithecium (Text-fig. 4 B) is from three to four cells in depth. The sutures dividing the wall into polygonal plates (Text-fig. 4 E) have already been referred to (*vide* p. 266). They are built up of tangentially elongated elements whose central cells are not thickened (Text-fig. 5 G). These cells form an almost straight line along which cleavage occurs when the perithecium explodes after drying. This liberates most of the polygonal



Text-fig. 4. Longitudinal sections through the perithecia of: A, *C. reniformis*. B, *C. purpurea*. C, *C. sulfurea*. Segments of the perithecial wall of D, *C. reniformis*. E, *C. purpurea*. F, *C. sulfurea*. (D–F drawn to the same scale with fine lines representing the position of sutures.)

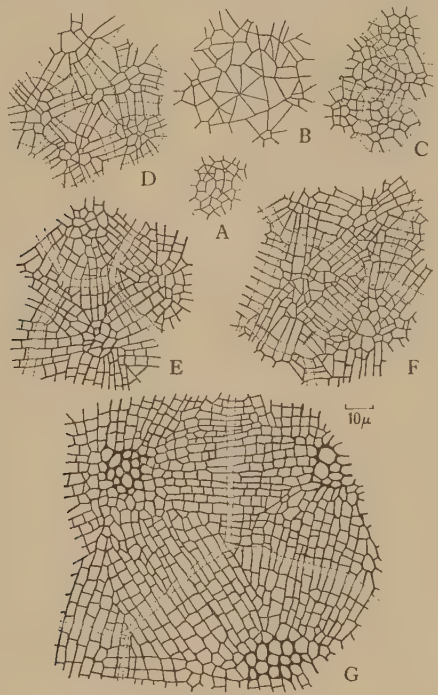
plates in the form of a flat disc of disjointed segments. The majority of the ascospores are dispersed by the explosion, but some remain in groups over the centre of the disc.

The young perithecia of this species seem to be invested by a purple villus. This is due to a pigment developed in the cells of the hyphae in the region of the perithecia and occurs characteristically in cultures exposed to daylight. The villus is not an integral part of each individual perithecium which produces only a few simple attachment hyphae. These seldom persist to maturity. That the purple pigment diffuses into the medium was noted by Shear (*loc. cit.* p. 125) who also

stated that the perithecia are dark purple. I have only occasionally observed this in cultures, and in field collections only that from oak possessed perithecia with definitely pigmented walls.

(3) *C. sulfurea.*

In *C. sulfurea* the ascogenous hyphae occupy practically the whole space within the perithecial wall (Text-fig. 3 K). There is only a thin layer of pseudoparenchymatous tissue lining the wall. The asci first appear towards the centre of the perithecium and are arranged at



Text-fig. 5. Stages in the development of sutures in the perithecia of *C. sulfurea* (A, C, E), *C. reniformis* (B, D, F) and *C. purpurea* (G). (Figures F and G are drawn from mature shattered perithecia but figure E is drawn from a young perithecium. All figures drawn to the same scale).

intervals along the ascogenous hyphae (Text-fig. 3 M). The almost mature perithecium (Text-fig. 4 C) has a relatively wide wall in which suture lines are clearly defined by layers of tangentially elongated thin-walled cells, in a manner similar to those of *C. purpurea* (Text-fig. 5 E). The whole perithecium is covered at this stage by a mass of interwoven, flexuose, thick-walled and sulphur-coloured

hyphae among which occur ordinary hyphae bearing conidia. The sulphur colour may be absent when the perithecia are grown in darkness, but it soon appears on exposure to light. At this time the ascogenous hyphae with their asci are still visible, but as the perithecium matures the former disorganise and only the younger of the latter remain intact, so that the perithecium is filled with clusters of ovoid, brown ascospores among which are a few eight-spored asci. As the perithecium enlarges, its apex becomes glabrous, while its base remains embedded in the mass of sulphur-coloured hyphae. Since the perithecia are formed in clusters, this sulphureous villus is very conspicuous both upon agar media and upon wood. When the perithecium shatters the wall breaks down along the suture lines, discharging the apical series of plates and leaving a basal cup of partially separated plates enclosing the spores.

The presence of definite suture lines in the perithecial wall of the three species of *Cephalotheca* is an important diagnostic character. Whether these sutures are visible, under low-power magnification, on the wall of the perithecium (as in *C. sulfurea* and *C. purpurea*) or not (as in *C. reniformis*) their presence can be demonstrated by suitable preparation. A comparison of their development in the three species shows that in each they are formed by the further growth of certain prismatic or triangular cells (Text-fig. 5 A, B) grouped round a polygonal central cell. The prismatic cells become elongated, and by transverse division (Text-fig. 5 C, D) produce radiating lamellae of thick-walled cells (Text-fig. 5 E, F, G). The central cell gives rise to an indurated plate of cells (Text-fig. 5 G). One or more cells, midway between the lamellae belonging to different plates, remain thin-walled and may represent meristematic cells. Collectively they represent the lines of suture along which the perithecium ruptures. These developmental stages, when considered along with other perithecial characters, indicate a close relationship between the three species.

IV. Discussion

(*a*) *The conidial stage*

Considering these three species on the evidence presented in this paper, it appears that the conidia described for *C. reniformis* and *C. purpurea* are homologous and fundamentally differ from the conidia described for *C. sulfurea*. I am of the opinion that a phialospore stage of *C. reniformis* and *C. purpurea* homologous with that of *C. sulfurea*—the type species of the genus—may yet be found.

(*b*) *The ascophorous stage*

The perithecial rudiments of *C. reniformis* and *C. purpurea* are of similar construction and differ only in size from those of *C. sulfurea*.

In the further differentiation of the perithecium, and particularly of the asci, *C. reniformis* and *C. purpurea* exhibit a closely parallel development which differs only in degree from that of *C. sulfurea*. According to Fuckel the diagnostic characters of the mature astomous perithecium are chiefly the fragile nature of the wall and the clustered arrangement of the asci at the ends of branched ascophorous hyphae. Von Höhnel (4) stated that besides being fragile the perithecia break up along characteristic sutures. The present investigation shows that the sutures in the wall of the perithecium of *C. purpurea* are precisely similar in structure and development to those in the perithecium of *C. sulfurea*. After emending the diagnosis to include von Höhnel's observation, and bearing in mind that *C. sulfurea* is taken as the type species, the only conclusion which can be drawn from the sum of the evidence presented here is that the three species should be generically related.

The coiled fundaments of the perithecium of the species described here are similar to those described by Brefeld for his *Penicillium glaucum* (*Carpenteles asperum* (Langeron) Shear (9)). The formation of the asci upon the ascogenous hyphae may be compared with that described for the *Aspergillus herbariorum* group or for *Penicillium Brefeldianum* (2). There is every reason to believe that *Cephalotheca* should be placed in relation to these genera and that it should be distinguished from them by the specialisation of the perithecium in regard to the liberation of the ascospores.

(*c*) *Synonymy*

The synonymy of *C. sulfurea* has been discussed by von Höhnel (5). He reviewed the position with regard to two species previously classified as Sphaeropsids and stated that *Aposphaeriopsis fusco-atra* Died. was *C. sulfurea*. He hazarded the view that *Fairmania singularis* Sacc. might be *C. sulfurea* and indicated that he thought *C. reniformis* merely a synonym for *C. sulfurea*. Petrak and Sydow (8), after comparing the type specimen of *Fairmania singularis* in Saccardo's herbarium with *C. sulfurea*, stated that they consider them synonymous. These reports indicate that the two Sphaeropsids should be regarded as species of *Cephalotheca*. Having had the advantage of studying a fungus which closely agrees with the description of *C. reniformis*, I am certain that this species is not synonymous with *C. sulfurea*. The latter has a *sulphur-coloured villus* surrounding the perithecium and ovoid ascospores measuring $6 \times 4\mu$. *C. reniformis* has a *glabrous* perithecium and reniform ascospores measuring $4–5 \times 3–3{\cdot}8\mu$. Diedicke describes *Aposphaeriopsis fusco-atra* with a *glabrous* fructification (200–250μ in diameter) containing spores measuring 4–5μ in diameter which are drawn as reniform (Pl. XII, fig. 5). These characters indicate that it is *C. reniformis* and not *C. sulfurea*. The spores of

Fairmania singularis are described as (Saccardo, *loc. cit.* p. 276): "Solae calcaneum exacte imitantis" (exactly like the heel of a slipper). At first hyaline, they later become sooty and measure 5·7μ in diameter. Since Petrak and Sydow consider *Fairmania singularis* a good *Cephalotheca*, these characters indicate that it should be held synonymous with *C. reniformis*.

V. Summary

A. *Diagnosis of* Cephalotheca

1. *Perithecia* astomous, simple, spherical, carbonaceous, fracturing at maturity along well-defined sutures; young perithecia sometimes covered by a coloured villus, in other species totally glabrous; on dry decayed wood, superficial or semi-immersed and free, or immersed and attached basally. Asci spherical or elliptical, on branched ascogenous hyphae, alternate or clustered, more or less sessile, eight-spored, evanescent. Ascospores conglobate, ovate or bean-shaped or reniform, continuous, hyaline to yellow or fuscous (light to dark brown in mass).

2. *Conidia* formed upon a white or slightly coloured mycelium. Conidia hyaline, continuous, either serial phialospores, ovate or broadly elliptical, or radulospores, falcate or suballantoid.

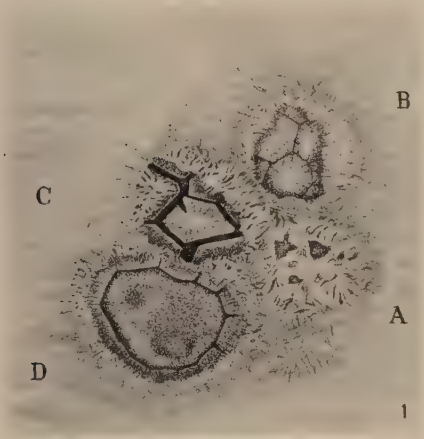
Type species: *C. sulfurea*.

B. *Key to the accepted species of* Cephalotheca

(*a*) *Ascospores ovate.*
Perithecia surrounded by a sulphureous villus.
Ascospores 6 × 4μ. Phialospores 3–5 × 2–3μ. ***C. sulfurea.***

(*b*) *Ascospores ± reniform.*
(i) Perithecia partly invested with a transitory purple coloured villus. Ascospores bean-shaped 2·3–3·0 × 1·5–2·0μ. Radulospores 3–4 × 1·5μ. ***C. purpurea.***
(ii) *Perithecia* totally glabrous, attached basally to the substratum. Ascospores reniform 4–5 × 3·0–3·8μ. Radulospores 4–5 × 1·5μ. ***C. reniformis.***

I desire to express my gratitude to Mr E. W. Mason, of the Imperial Mycological Institute, Kew, not only for suggesting this investigation and for providing material but also for his continued help and criticism throughout its course. The kind assistance of Dr C. L. Shear, of Washington, U.S.A., made it possible to place the type species of *Fragosphaeria* exactly. To Mr W. B. Grove, of Birmingham, I am deeply indebted for the collection of *C. sulfurea* which rendered a thorough comparison between the type species of the two genera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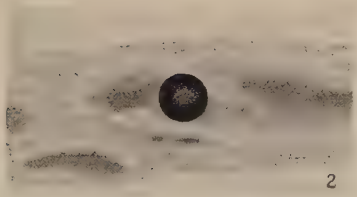
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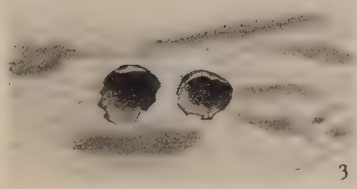
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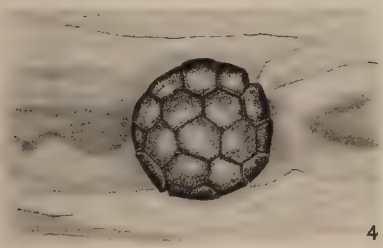
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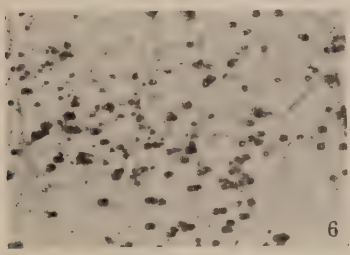
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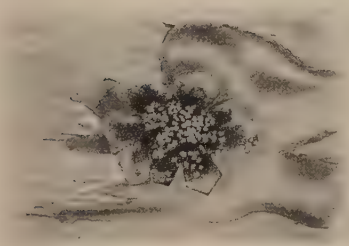
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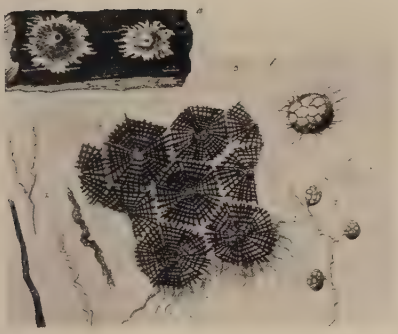

Fig. 1

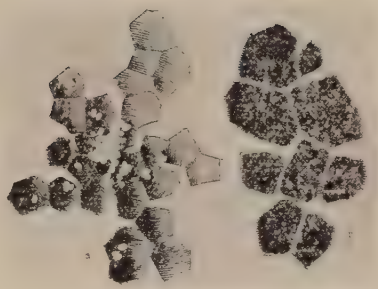

Fig. 2

Fig. 3

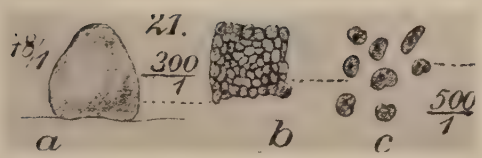

Fig. 5

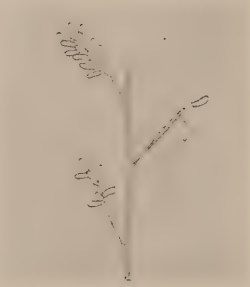

Fig. 4

possible. My thanks are also due to Mr J. Ramsbottom and Miss E. M. Wakefield for affording facilities for the inspection of Herbarium material.

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EXPLANATION OF PLATES XI AND XII

Plate XI

Fig. 1. Perithecia of *Cephalotheca sulfurea* in various stages of development.
Fig. 2. Mature perithecium of *C. reniformis* on beech wood.
Fig. 3. Shattered perithecia of *C. reniformis*.
Fig. 4. Mature perithecium of *C. purpurea* showing suturing.
Fig. 5. Shattered perithecium of *C. purpurea* showing clustered groups of ascospores.
Fig. 6. Perithecia of *C. reniformis* developed on a culture of beech wood.
Fig. 7. Perithecia of *C. purpurea* on a similar culture.

Plate XII

Reproductions of drawings of species of *Cephalotheca*.
Fig. 1. *C. sulfurea* (Berkeley and Broome, 1878).
Figs. 2–4. *C. purpurea* (Shear, 1923).
Fig. 5. *Aposphaeriopsis fusco-atra* (Diedicke, 1913).
For further explanation see text.

While this paper was in press an extensive collection of *Cephalotheca sulfurea* was made at Birmingham on structural timber which had been covered by ashes. The mycelium bore both phialospores and mature perithecia. There now seems to be little doubt that this species is a "domestic mould" occurring superficially upon a variety of woods.